Kidney International, Vol. 45 (1994), pp. 537–543

Angiotensin converting enzyme inhibitor modulates glomerular function and structure by distinct mechanisms

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Angiotensin converting enzyme inhibitor modulates glomerular function and structure by distinct mechanisms. Rats with puromycin aminonucleoside (PAN) nephrosis were given either angiotensin I converting enzyme inhibitor (ACEI), angiotensin II type I receptor antagonist (Ang IIRA), or no treatment for four weeks and were then monitored for an additional 12 weeks. In untreated PAN rats, proteinuria reached a maximum at two weeks (271 ± 38 mg/day). Proteinuria in this early phase was markedly attenuated by ACEI (96 ± 35 mg/day, $P < 0.01$), but unaffected by Ang IIRA (306 ± 34 mg/day). Acute administration of a bradykinin antagonist substantially dampened the antiproteinuric effect of ACEI in PAN rats, resulting in an average increase in proteinuria of 41 ± 14% in ACEI-treated rats ($P < 0.05$, ACEI vs. ACEI + bradykinin antagonist). Acute phase therapy for four weeks with ACEI or Ang IIRA did not attenuate subsequent glomerulosclerosis. Separate groups of PAN rats with similar degree of glomerulosclerosis, assessed at 16 weeks after PAN by renal biopsy, were then treated as follows: ACEI [50 mg/liter drinking water (DW), or 200 mg/liter DW], Ang IIRA (20 mg/liter DW, or 80 mg/liter DW) or no treatment, starting after renal biopsy. Whereas glomerulosclerosis increased from biopsy to autopsy at 28 weeks with emergence of low grade proteinuria in untreated PAN rats, proteinuria was absent and glomerulosclerosis was ameliorated or reversed in ACEI and Ang IIRA groups. The results indicate that the early phase proteinuria of PAN nephropathy is independent of Ang II, and that the antiproteinuric effect of ACEI is, at least in part, channeled through activation of bradykinin, whereas the subsequent progression of glomerulosclerosis is caused by a mechanism involving endogenous Ang II actions. The results also indicate that the therapeutic effect of a treatment on glomerulosclerosis cannot be predicted from its earliest effects on proteinuria.

Recently, we and others reported that not only angiotensin I converting enzyme inhibitor (ACEI) [1–4], but also specific angiotensin II type 1 receptor antagonist (Ang IIRA) [5, 6] is highly effective in ameliorating the progression of glomerulosclerosis in a remnant kidney model. The results established a role for endogenous angiotensin II in the pathogenic process of glomerular sclerosis in this model [5, 6]. In the present study, we investigated the puromycin aminonucleoside (PAN) nephrosis model, which more closely resembles human chronic renal diseases [7, 8]. Initial nephron number is normal and injury is diffuse in this model, in marked contrast to the renal ablation model characterized by a reduced number of nephrons that are initially structurally normal with subsequent adaptive responses of glomerular growth, glomerular hypertension and hyperfiltration [9, 10] preceding the development of glomerulosclerosis. Intravenous injection of PAN causes rats to develop massive proteinuria and glomerular lesions that are ultrastructurally and biochemically identical to those of human minimal change disease. The initial nephrotic syndrome then abates spontaneously and proteinuria virtually disappears. However, without additional PAN administration, proteinuria recurs and glomerular lesions develop which have a remarkable resemblance to the mesangial expansion and glomerulosclerosis seen in a variety of human chronic renal diseases [7, 8]. As in the remnant model, ACEI is effective in attenuating the progressive deterioration of glomerular architecture in the PAN model [7, 8].

Thus, we postulated that treatment with Ang IIRA in this model would test the pathogenic importance of endogenous Ang II in a setting of progressive glomerular damage relevant to human disease. In addition, this study clarified some unresolved issues, namely whether the proteinuria-reducing effect of a therapeutic measure, that is, ACEI, represents its capacity to protect kidneys from progressive structural damage.

Methods

Experimental groups

All experiments were performed on adult male Munich-Wistar rats weighing 180 to 240 g. Body weights were not different among groups at the beginning of the experiment. The rats had free access to standard rat chow and tap water.

Acute nephrotic phase

Under sodium pentobarbital (Nembutal, 30 mg/kg body wt) anesthesia, the right jugular vein was cannulated and injected with 42 mg/kg body wt puromycin aminonucleoside (PAN; Sigma Chemical Co., St. Louis, Missouri, USA) dissolved in 3 ml of saline, given as a single injection over five minutes [8]. After recovery, rats were divided into specific treatment groups. Group 1 (PAN control, $N = 10$) received no therapy. Group 2 (PAN + ACEI, $N = 9$) was treated with angiotensin I converting enzyme inhibitor (ACEI, enalapril maleate, Merck Research Laboratories, Rahway, New Jersey, USA, "high dose" 200 mg/liter drinking water, DW) for the first four weeks after PAN injection (defined as acute nephrotic phase). Group 3

Received for publication June 28, 1993
and in revised form September 2, 1993
Accepted for publication September 2, 1993

(PAN + Ang IIRA, $N = 7$) was treated with angiotensin II receptor antagonist (Ang IIRA, L-158,809, Merck, "high dose" 80 mg/liter DW) for the first four weeks. The Ang IIRA used is a new angiotensin II type 1 receptor antagonist which shows high affinity and selectivity for the type 1 receptor with no agonist activity. The drug is orally active and with long-lasting effects, over six hours after a single dose in the rat [11–13]. These doses were four times the minimum required for control of systemic blood pressure in our previous study in the remnant kidney model [5]. In pilot studies, this high dose of ACEI, in contrast to minimum antihypertensive dose ($N = 3$, 50 mg/liter DW), was found to ameliorate proteinuria in the acute phase. After four weeks, all three groups were monitored without further treatment for an additional 12 weeks. At 2, 8 and 16 weeks, all rats were housed in metabolic cages to obtain 24-hour urine collections. All rats were sacrificed at 16 weeks after PAN to examine renal morphology.

To explore a possible role of bradykinin in ACEI effects on proteinuria, a separate group of rats treated with PAN ($N = 8$) or PAN + ACEI ($N = 8$) for two weeks were anesthetized and studied before and after acute treatment with a bradykinin antagonist, Hoe 140 (0.1 mg/kg body wt, simultaneous single i.v. and single s.c. doses, Hoechst, Frankfurt, Germany). This dose of bradykinin antagonist has previously been shown to be highly specific *in vivo*, completely preventing systemic hypotension in response to exogenous bradykinin after one hour and with only minimal hypotension (~5% decrease in blood pressure) two hours following subcutaneous administration [14]. Whole kidney function (described below) and proteinuria, from timed collections of urine from catheterized ureters, were assessed at baseline and one hour after Hoe 140 administration.

Chronic glomerulosclerosis phase

Rats were injected with PAN as above. Three rats did not show the typical response to PAN injection (urinary protein excretion < 50 mg/day 2 weeks after PAN) and were excluded from the study. At 16 weeks, open renal biopsy of the right kidney was performed under pentobarbital anesthesia via a midline abdominal incision to assess histologic changes as previously described [1]. Based on similar levels of renal injury in the renal biopsy (specified below), rats were grouped and treatment started within two days of biopsy. Group 4 (PAN control; $N = 10$) received no therapy. Group 5 (PAN + ACEI) was treated with ACEI, either "low dose" (Group 5a; 50 mg/liter DW, $N = 8$) or "high dose" (Group 5b; 200 mg/liter DW, $N = 8$). Group 6 (PAN + Ang IIRA) was treated with Ang IIRA, either "low dose" (Group 6a; 20 mg/liter DW, $N = 7$) or "high dose" (Group 6b; 80 mg/liter DW, $N = 8$). "Low dose" was the minimum dose required to decrease systemic blood pressure in our previous study of remnant kidney model [5]. Group 7 (normal control, $N = 7$) consisted of age-matched normal animals that underwent open renal biopsy and were sacrificed 12 weeks after the biopsy. At 2, 8, 16 and 28 weeks after PAN injection, all rats were housed individually in metabolic cages to obtain 24-hour collections of urine for determinations of protein, creatinine clearances (16 and 28 weeks) and urinary electrolytes (28 weeks). Systolic blood pressure was measured in the conscious state by tail cuff method [15] before each 24-hour urine collection. Rats were sacrificed at 28 weeks after assessment of renal function and collection of plasma for creatinine, electrolytes and triglycerides. Tissues were examined as described below and morphology was compared between biopsy and autopsy specimens in the same animals.

Whole kidney function and micropuncture studies for the chronic phase study

Group 4 to 7 rats were anesthetized with Inactin (Byk, Gulden Konstanz, Germany; 100 mg/kg body wt, i.p.) at 28 weeks for measurements of kidney function, as previously described [16]. Briefly, following tracheotomy, indwelling polyethylene catheters (PE-50, Clay Adams, Parsippany, New Jersey, USA) were placed into the left jugular vein for infusion of plasma, inulin and para-aminohippurate (PAH) and the left femoral artery was cannulated to monitor mean systemic arterial pressure (MAP) as previously described [17]. Two consecutive timed collections of urine samples were obtained through ureteral cannulations for determination of urine flow rate, inulin and PAH concentrations. Arterial blood was collected into capillary tubes for determinations of plasma inulin and PAH concentrations at the midpoint of urine collection. Glomerular filtration rate (GFR) and renal plasma flow rate (RPF) were then estimated by inulin and PAH clearance, respectively. In addition, glomerular capillary hydraulic pressure (P_{GC}) was measured in eight rats (Group 4), four rats (Group 5a), six rats (Group 5b), four rats (Group 6a), six rats (Group 6b) and seven rats (Group 7) with a continuous recording, servo-nulling pressure system (model 4A, Instrumentation for Physiology and Medicine, San Diego, California, USA) as previously described [17].

Histological studies for acute nephrotic phase and chronic glomerulosclerosis phase treatments

Light microscopic studies were performed on the autopsy kidney specimens (16 weeks) for the acute nephrotic treatment phase study and on the biopsy (16 weeks) and autopsy (28 weeks) kidney specimens from the same rat for the chronic glomerulosclerosis phase study. Tissue was immersion fixed in 10% buffered formalin, routinely processed, paraffin embedded and 4 μm sections cut and stained with periodic acid-Schiff. Histological examination was performed without knowledge of the treatment protocol. A semiquantitative score (sclerosis index, SI) modified from the method of Raij, Azar and Keane was used to evaluate the degree of glomerular sclerosis [18]. Sclerosis was defined as collapse and/or obliteration of the glomerular capillary tuft accompanied by hyalin material, increase of matrix and/or adhesion of the tuft to Bowman's capsule. On a single section of the kidney, glomerular sclerosis was assessed by examining all glomeruli and severity of sclerosis for each glomerulus was graded from 0 to 4+. Early lesions were graded from 0.25 to 1+ as follows: a 0.25+ lesion represented adhesions of the tuft to Bowman's capsule, a 0.5+ lesion represented sclerosis of up to 10% of the glomerulus and 1+ represented sclerosis of 10 to 25% of the glomerulus, while 2+, 3+ and 4+ represented sclerosis of 25 to 50, 50 to 75 and 75 to 100% of the glomerulus, respectively. A whole kidney sclerosis index was obtained by averaging scores from all glomeruli on one section.

The presence or absence of mesangial expansion was also evaluated in each glomerulus on a single section, as follows: A

Table 1. Proteinuria and glomerulosclerosis in acute phase PAN rats

	Proteinuria *mg/day*				
	2 weeks	8 weeks	16 weeks	S.I.	Mesangial expansion %
Group 1 ($N = 10$)	271 ± 38	12 ± 2	15 ± 3	0.27 ± 0.02	30 ± 6
Group 2 ($N = 9$)	96 ± 35[a]	8 ± 1	10 ± 2	0.26 ± 0.02	30 ± 4
Group 3 ($N = 7$)	306 ± 34	10 ± 2	18 ± 3	0.25 ± 0.02	34 ± 2

Results are presented as mean ± SE. Abbreviation is: SI, sclerosis index.

[a] $P < 0.01$ versus Group 1 and Group 3

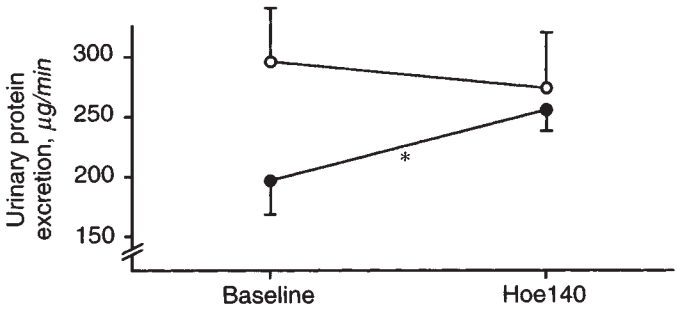

Fig. 1. *The effect of the bradykinin antagonist Hoe 140 on proteinuria in acute phase PAN rats.* The decreased proteinuria in PAN + ACEI rats increased significantly with Hoe (●) (*$P < 0.05$). In contrast, Hoe did not change proteinuria in rats treated with PAN only (○). Mean arterial pressure did not change with Hoe 140 in PAN + ACEI rats or PAN rats. SE is indicated by bar.

positive score was given for glomeruli with globally (>50% of the glomerular tuft) increased mesangial area. No score was given for glomeruli without mesangial expansion or only focally (≤50% of glomerular tuft) increased mesangial area. This scoring was based on the segmental mesangial expansion often seen in normal control rat glomeruli. These data are expressed for the whole kidney as % mesangial expansion, representing the % of positive glomeruli per total glomeruli.

More than 25 glomeruli were analyzed in each biopsy specimen and a minimum of 50 glomeruli was analyzed in the autopsied kidney.

Analytical

Urinary protein was assessed by the Coomassie brilliant blue method [19]. Plasma sodium, plasma potassium, urinary sodium and urinary potassium were measured by flame photometry. Plasma creatinine was measured by a "Monarch Chemistry System" centripetal analyzer (Instrumentation Laboratory, Inc., Milan, Italy) by the modified Jaffe reaction, alkaline picrate without Lloyds method, and plasma triglycerides were measured by a modification of the method of Bucolo and David [20]. Inulin concentrations in plasma and urine were determined by the macro anthrone method [21]. PAH concentrations in plasma and urine were assayed by the method of Bratton and Marshall, modified by Smith et al [22].

Statistical methods

Results are expressed as mean ± standard error (SE). Comparisons between two time periods in the same animals or between groups paired based on renal biopsy data were made using the paired *t*-test for parametric data or Wilcoxon signed-ranks test for nonparametric data. Nonparametric data from multiple groups were tested using the Kruskall-Wallis test followed by Mann-Whitney U test with modification by Bonferroni's method. Parametric data from multiple groups were compared using one-way analysis of variance (ANOVA) followed by *t*-test with modification by Bonferroni's method.

Results

Acute nephrotic phase

Effect of ACEI or Ang IIRA on urinary protein excretion and morphology. Table 1 shows urinary protein excretion rate in Groups 1 to 3. Pilot experiments showed that i.v. infusion of PAN induced massive proteinuria within five days, which peaked at two weeks and then normalized at eight weeks. Thus, we chose two weeks after PAN to examine the proteinuria level. PAN rats (Group 1) showed massive proteinuria (271 ± 38 mg/day) at 2 weeks. In contrast, in ACEI-treated rats (Group 2), this early phase proteinuria was markedly attenuated (96 ± 35 mg/day, $P < 0.01$ vs. Group 1). However, Ang IIRA treatment (Group 3) did not affect proteinuria (306 ± 34 mg/day, $P < 0.01$ vs. Group 2, P = NS vs. Group 1). By eight weeks after PAN infusion, urinary protein excretion rate decreased to almost normal levels in all three groups without significant change by 16 weeks (Table 1). Degree of sclerosis at 16 weeks was similar among Groups 1 to 3 (SI 0.27 ± 0.02, 0.26 ± 0.02 and 0.25 ± 0.02, respectively). Further, the degree of mesangial expansion was also similar among the three groups (30 ± 6%, 30 ± 4% and 34 ± 2%, respectively). Thus, acute phase therapy for four weeks with ACEI or Ang IIRA did not affect the subsequent progression over the next 12 weeks of glomerular injury. Body weights were not different among groups at completion of studies (body wt range 289 to 345 g).

Effect of bradykinin antagonist on proteinuria in acute phase. Differing effect of decreasing proteinuria in PAN rats by inhibiting Ang II action via ACEI or Ang IIRA indicated that non-Ang II actions of ACEI modulated acute proteinuria. Since ACEI is known to potentiate bradykinin activity, we examined the effects of a specific bradykinin antagonist on ACEI-induced decrease in acute phase proteinuria. Inhibition of bradykinin activity increased urinary protein excretion on average 41 ± 14% in each ACEI-treated rat (197 ± 29 vs. 256 ± 18 μg/min, $P < 0.05$; Fig. 1). This effect occurred without significantly affecting MAP, RPF and GFR (91 ± 2 vs. 92 ± 2 mm Hg, 1.08 ± 0.28 vs. 1.16 ± 0.34 and 0.21 ± 0.04 vs. 0.25 ± 0.05 ml/min, P = NS, ACEI baseline vs. Hoe 140, respectively). Bradykinin antagonist did not affect urinary protein excretion in PAN rats not treated with ACEI (296 ± 44 vs. 274 ± 46 μg/min, P = NS), nor did it affect MAP, RPF and GFR (101 ± 5 vs. 101 ± 6 mm Hg, 1.21 ± 0.19 vs. 1.15 ± 0.23 and 0.32 ± 0.04 vs. 0.38 ± 0.06 ml/min, P = NS, respectively).

Chronic glomerulosclerosis phase

Effect of ACEI or Ang IIRA on urinary protein excretion. Table 2 shows urinary protein excretion in Groups 4 to 7. There were no differences in proteinuria at 2, 8 or 16 weeks after PAN among the groups. However, in Group 4 without treatment,

Table 2. Urinary protein excretion in chronic phase PAN rats

	Proteinuria *mg/day*			
	2 weeks	8 weeks	16 weeks	28 weeks
Group 4 ($N = 10$)	179 ± 22	15 ± 3	20 ± 5	47 ± 9[a]
Group 5a ($N = 8$)	159 ± 25	7 ± 1	9 ± 2	12 ± 6
Group 5b ($N = 8$)	197 ± 31	10 ± 2	14 ± 4	9 ± 3
Group 6a ($N = 7$)	172 ± 41	11 ± 3	13 ± 5	8 ± 3
Group 6b ($N = 8$)	215 ± 32	10 ± 4	19 ± 7	9 ± 2
Group 7 ($N = 7$)	N/D	N/D	6 ± 1	15 ± 2

Results are presented as mean ± SE. Abbreviation is: N/D—not done.
[a] $P < 0.05$ versus Group 5a, 5b, 6a, 6b and 7

proteinuria increased significantly at 28 weeks after PAN compared to the normal age-matched control rats (Group 7) ($P < 0.05$). In contrast, ACEI- and Ang IIRA-treated rats (Groups 5a, 5b, 6a and 6b), showed complete absence of recurrent proteinuria ($P < 0.05$, compared with Group 4).

Serum and urinary electrolytes and serum triglycerides. Plasma and urinary electrolytes and serum triglyceride values were measured at 28 weeks after PAN injection. Rats given ACEI or Ang IIRA had normal levels of plasma sodium and potassium, and no change in urinary excretion of sodium and potassium (data not shown). Thus, high dose ACEI or Ang IIRA, as well as low dose ACEI or Ang IIRA, had no untoward effects on these electrolyte values. Triglycerides were not increased in PAN-treated rats at 28 weeks (range 55 ± 3 to 68 ± 11 mg/dl) compared to normal control Group 7 (66 ± 9 mg/dl).

Whole kidney function studies and micropuncture. Table 3 shows results of systemic blood pressure and renal function studies. At 16 weeks after PAN, systemic blood pressure levels in PAN rats (Group 4) were similar to normal control (Group 7). Systemic pressure in PAN rats at 28 weeks after PAN continued to be normal. Glomerular capillary hydraulic pressure levels were also similar between PAN rats and normal control rats. High dose ACEI and low and high dose Ang IIRA decreased the systemic blood pressure and mean arterial pressure below normal. High dose ACEI and Ang IIRA also decreased the glomerular pressure below that seen in normal rats.

There was no difference among the groups in creatinine clearance at the onset of therapy at 16 weeks and no difference in clearances of creatinine, inulin or PAH at 28 weeks. Body weight gains during treatment in Groups 6a and 6b were significantly lower than in Group 4, PAN treated rats ($P < 0.05$).

Glomerular morphology of biopsy (Bx) and autopsy specimens. The degrees of glomerular sclerosis and mesangial expansion were not different among the groups at the onset of therapy at 16 weeks, as dictated by the study design (SI; 0.29 ± 0.02 in Group 4, 0.30 ± 0.05 in Group 5a, 0.32 ± 0.05 in Group 5b, 0.29 ± 0.04 in Group 6a and 0.38 ± 0.06 in Group 6b, respectively, P = NS; mesangial expansion; 36 ± 6% in Group 4, 37 ± 7% in Group 5a, 40 ± 5% in Group 5b, 39 ± 5% in Group 6a and 46 ± 7% in Group 6b, respectively, P = NS). Age-matched normal control rats (Group 7) had SI 0.09 ± .01 and mesangial expansion 24 ± 2% at biopsy, with similar morphology at autopsy at 28 weeks (SI 0.11 ± .01, mesangial expansion 22 ± 3%). Figure 2A presents the percent change in SI and mesangial expansion from biopsy to autopsy specimens in ACEI-treated rats. In Group 4 PAN rats without treatment, the degree of sclerosis progressed in all rats during the twelve weeks with an average increase in SI of 69 ± 17% from 16 to 28 weeks after PAN. In rats given ACEI, low dose ACEI attenuated substantially the degree of progression of sclerosis with average increase in SI of only 13 ± 21% (P = NS, Bx vs. autopsy; $P < 0.05$, vs. Group 4). High dose ACEI further attenuated the progression: SI and mesangial expansion decreased significantly from biopsy to autopsy (SI; −30 ± 7%, $P < 0.05$, Bx vs. autopsy; $P < 0.01$, vs. Group 4 and $P < 0.05$, vs. Group 5a, mesangial expansion; −60 ± 7%, $P < 0.001$, Bx vs. autopsy; $P < 0.05$, vs. Group 4). Figure 2b shows the percent change in SI and mesangial expansion from biopsy to autopsy specimens in Ang IIRA-treated rats. Low dose Ang IIRA attenuated substantially the degree of progression of sclerosis with average change in SI of −7 ± 16% (P = NS, Bx vs. autopsy; $P < 0.05$, vs. Group 4). High dose Ang IIRA further attenuated the progression with significant decreases in SI and mesangial expansion (SI; −41 ± 8%, $P < 0.01$, Bx vs. autopsy; $P < 0.01$, vs. Group 4 and $P < 0.05$, vs. Group 6a, mesangial expansion; −68 ± 5%, $P < 0.002$, Bx vs. autopsy; $P < 0.05$, vs. Group 4). In 15 of 16 rats (94%) treated with "high dose" ACEI or Ang IIRA, mesangial expansion and glomerulosclerosis were decreased with less severe lesions at autopsy than at biopsy in these same rats. Thus, high dose ACEI and Ang IIRA had significantly greater beneficial effects on glomerulosclerosis compared to low dose of each of the treatments.

Discussion

Proteinuria has been widely used as an index of disease activity in a variety of renal injuries. Changes in magnitude of proteinuria in turn have been used as a parameter which reflects the efficacy of a variety of therapeutic interventions, including dietary manipulations and antihypertensive medications [23, 24]. It should be noted that the specific glomerular capillary wall structural lesions which are linked to proteinuria have not been established [25–28], although it is assumed that this unknown structural lesion parallels the morphologically obvious lesions, such as glomerular sclerosis. Of interest, a previous study in a rat model of chronic progressive glomerular sclerosis demonstrated that the proteinuria originates primarily from glomeruli free of sclerosis [26]. Moreover, the sieving defect in these light microscopically intact glomeruli was largely corrected when the prevailing abnormally high intraglomerular pressure was normalized by acute administration of a vasodilator [26]. Assessment of the glomerular capillary pores revealed increased number of nonselective large pores during massive proteinuria at baseline and a reduction in these pores toward, but not to, the normal control level by normalization of glomerular pressure. Thus, although the precise structural abnormality of the glomerular capillary wall responsible for the sieving defect of chronic glomerular diseases remains unknown, and may even vary in different diseases, the previous studies [26–29] establish the notion that the abnormality consists of two separate components, one acutely reversible (or 'functional') and the other

Table 3. Whole kidney function and glomerular capillary pressure in chronic phase PAN rats

	SBP *mm Hg*		MAP	P_{GC} *mm Hg*	C_{Cr} *ml/min*		C_{In}	C_{PAH}
	16W	28W	*mm Hg*	*N*	16W	28W	*ml/min*	
Group 4	118 ± 3	127 ± 3	110 ± 4	56 ± 2 (8)	1.04 ± 0.09	1.03 ± 0.23	1.02 ± 0.05	4.33 ± 0.26
Group 5a	123 ± 3	128 ± 3	108 ± 5	54 ± 5 (4)	1.30 ± 0.12	1.13 ± 0.21	0.98 ± 0.09	5.61 ± 0.63
Group 5b	115 ± 4	101 ± 4[a,b]	88 ± 3[c]	46 ± 1[e] (6)	1.08 ± 0.13	1.37 ± 0.27	1.09 ± 0.11	4.58 ± 0.45
Group 6a	122 ± 4	100 ± 3[a,b]	91 ± 4[d]	49 ± 6 (4)	1.34 ± 0.21	1.16 ± 0.23	1.01 ± 0.16	4.53 ± 0.62
Group 6b	114 ± 3	97 ± 4[a,b]	91 ± 5[c]	45 ± 1[e] (6)	1.00 ± 0.08	1.20 ± 0.16	0.93 ± 0.05	5.45 ± 0.81
Group 7	114 ± 2	118 ± 4	100 ± 4	53 ± 3 (7)	1.11 ± 0.06	1.25 ± 0.13	1.03 ± 0.07	4.68 ± 0.42

Results are presented as mean ± SE. Abbreviations are: SBP, systolic blood pressure; C_{Cr}, creatinine clearance; C_{In}, inulin clearance; C_{PAH}, para-aminohippurate clearance; MAP, mean arterial pressure; P_{GC}, glomerular capillary pressure.

[a] $P < 0.01$ versus Group 4
[b] $P < 0.05$ versus Group 7
[c] $P < 0.01$ versus Group 4
[d] $P < 0.05$ versus Group 4
[e] $P < 0.05$ versus Group 4

acutely nonreversible (or 'chronically fixed'). Whether these functional and structural components are pathogenetically linked, and attributable to Ang II *per se*, was investigated in these studies.

Our results show that the acute phase therapy with either ACEI or Ang IIRA does not affect the development of glomerulosclerosis, although ACEI, in contrast to Ang IIRA, was effective in decreasing the acute nephrotic phase proteinuria. Although the possibility that progression of glomerulosclerosis beyond 16 weeks might be affected differentially by these acute treatments has not been specifically excluded, the similar injury 12 weeks after therapy suggests that subsequent progression also will be parallel. Previous investigations, using repeated PAN injections and/or lower doses of ACEI, showed no effect of ACEI on massive proteinuria in the acute nephrotic stage of PAN nephrosis [7, 30, 31]. Our pilot study confirmed that low dose ACEI does not attenuate massive proteinuria in the acute nephrotic stage, and contrasts the effectiveness of high dose ACEI in reducing proteinuria. These findings together with the salient and prompt effect of the bradykinin antagonist to dampen the antiproteinuric effect of ACEI argues strongly that the antiproteinuric effect of ACEI during early stage is 'functional' in nature. It also supports that ACEI's ability to reduce proteinuria at this early stage is driven by angiotensin-independent mechanism(s). Recently, Hutchison, Webster and Jaffa showed that ACEI decreased proteinuria and also increased renal kallikrein mRNA in nephrotic rats with Heymann nephritis [32]. Our results with the bradykinin antagonist further indicate that ACEI's early effect on proteinuria largely reflects its well known ability to inhibit kininase activity, thereby augmenting the local level of bradykinin [33], although the possibility that other vasoactive substances might contribute to lesser degree exists. It is possible that higher doses or longer duration of bradykinin antagonism may result in even greater reversal of the reduced proteinuria in ACEI-treated rats.

How, then, does bradykinin lead to correction of the sieving defect in the glomerular capillary wall in such a short period of time? Bradykinin decreased resistances of both afferent and efferent arterioles in an earlier study [34]. However, recent *in vitro* [35] and *in vivo* [36] studies found that bradykinin acts as a selective efferent arteriolar vasodilator. In rats with severe vasoconstriction of the efferent arteriole, ACEI induced a profound dilation of the arteriole and a marked fall in glomerular pressure, phenomena readily abolished by simultaneous administration of a specific bradykinin antagonist [36]. It is therefore conceivable that the ACEI-activated mechanism of bradykinin to correct the sieving defect in early proteinuria is channeled through its ability to reduce glomerular pressure and the stretch force imposed upon the glomerular capillary wall. Such reduction in stretch force can reduce the number of large non-selective pores [26, 27]. ACEI may also correct the impaired charge barrier function of the glomerular capillary wall seen in PAN nephrosis [37].

In contrast to the effects seen in the early stage, ACEI and Ang IIRA were equally effective in protecting glomeruli from progressive glomerular sclerosis and from 'chronically fixed' sieving defect of the glomerular capillary wall. Ang II has known effects on growth and matrix production in addition to its vasoconstrictor effects [38, 39]. Previous data showed that ACEI not only inhibits rapid maturational growth, but also glomerulosclerosis associated with aging [40, 41]. Since mesangial matrix production is a key element linked to glomerulosclerosis, and angiotensin II promotes matrix accumulation [38], the decreased matrix in ACEI-treated rats versus age-matched controls suggests that ACEI also inhibits the normal increase in matrix seen with maturational growth and aging. Our previous data showed for the first time the potential greater therapeutic benefit of ACEI, in doses higher than those required for blood pressure control, on structural injury in progressive renal disease [1]. These same high doses were also beneficial in the non-hypertensive PAN model in the current study. Of interest, it has been proposed that lower than normal systemic blood pressure can ameliorate structural injury, although a mechanism for such an effect has not been clarified [42]. Recently, Heeg et al showed that the long-term antiproteinuric effect of ACEI is not influenced by acute changes in systemic and renal

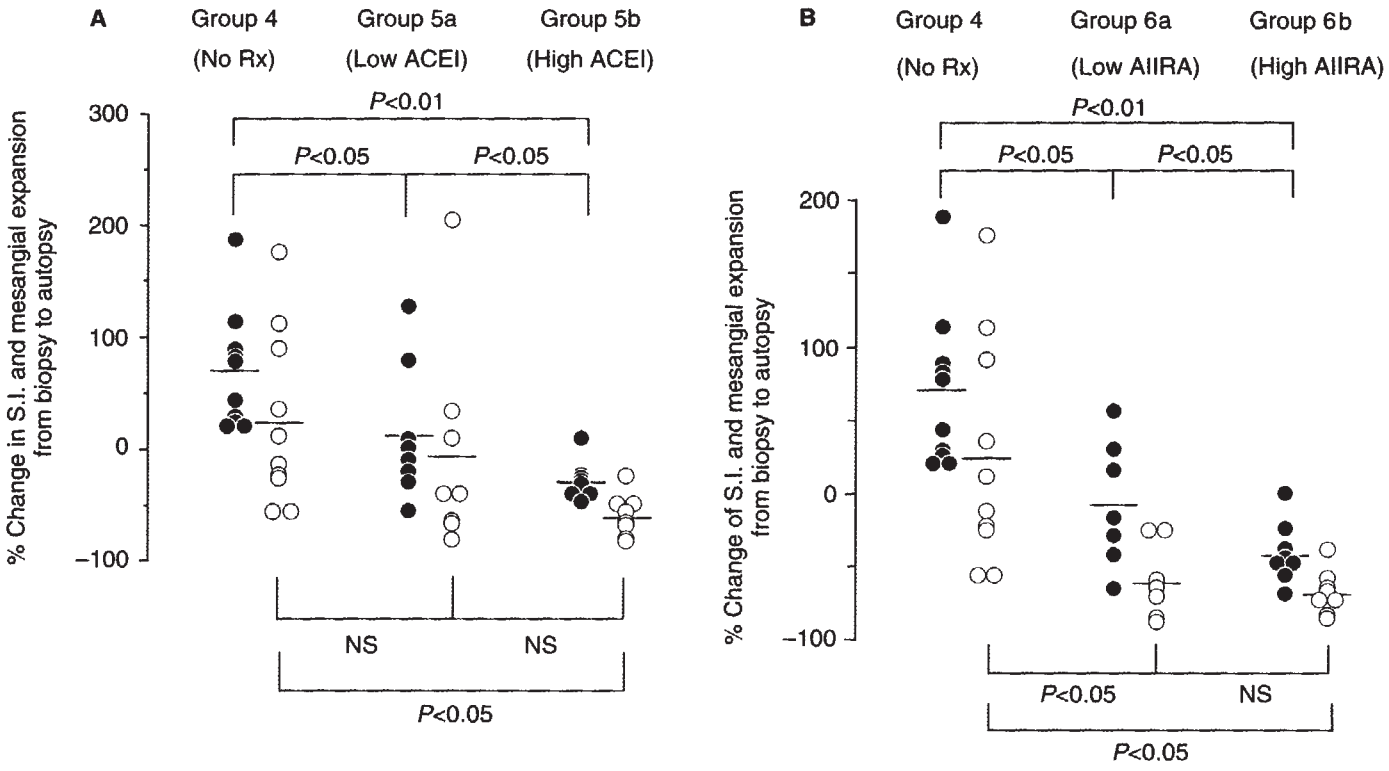

Fig. 2A. *Percent changes of sclerosis index (●) and mesangial expansion (○) from biopsy to autopsy specimens in rats with no treatment (No Rx) (Group 4), "low dose" ACEI (Group 5a) and "high dose" ACEI (Group 5b), and B. in rats with no treatment (no Rx) (Group 4), "low dose" Ang IIRA (Group 6a) and "high dose" Ang IIRA (Group 6b).* Each closed circle represents the percent change of sclerosis index at biopsy versus autopsy in each rat. Each open circle represents the percent change of mesangial expansion at biopsy versus autopsy in each rat. The mean value is indicated by bar.

hemodynamics [43]. ACEI or Ang IIRA effect in reducing the chronic phase proteinuria therefore likely reflects at least in part their ability to ameliorate a structural defect in the glomerulus in the PAN model. Unlike early proteinuria, the glomerular sclerosis and the attendant proteinuria in the chronic phase are driven by mechanism(s) involving endogenous angiotensin II actions. Thus, these studies show for the first time that ACEI's functional (that is, acute effects on proteinuria) and structural (that is, chronic effects on glomerulosclerosis) effects are largely unrelated, the former related to bradykinin and the latter to angiotensin II actions.

In view of these separate pathogenic mechanisms acting in early versus late phase of the progressive chronic disease, it is clear that the efficacy of a given experimental treatment to protect glomeruli from glomerulosclerosis cannot be predicted from its early effect on proteinuria. For example, a powerful angiotensin-independent agent capable of reducing early proteinuria may not protect glomeruli from (angiotensin II-dependent) glomerular sclerosis. Likewise, the potency of angiotensin inhibitors, like Ang IIRA, to prevent glomerular sclerosis may be underestimated by their apparent ineffectiveness to decrease early proteinuria soon after initiation of treatment. For these reasons it appears imperative to firmly establish the efficacy of ACEI in human disease based on both long-term functional and morphological data.

Finally, we are also intrigued by the finding that amelioration of nephrotic phase proteinuria in PAN rats by short-term ACEI treatment alone did not affect the subsequent progressive glomerulosclerosis (Group 2). Thus, both Ang IIRA-treated rats with massive early proteinuria and ACEI-treated rats without early nephrotic syndrome eventually developed similarly severe glomerular sclerosis. The results suggest that proteinuria *per se* has a limited role in the development of glomerulosclerosis, although studies with longer duration of nephrotic proteinuria are necessary.

Acknowledgments

These studies were supported in part by National Institutes of Health Grants DK42131 and DK44757. Portions of these studies were presented at the annual meeting of the American Society of Nephrology in Baltimore in December of 1992 and published in abstract form (*J Am Soc Nephrol* 3:618, 1992). Dr. Fogo is a recipient of the Clinician Scientist Award from the American Heart Association. Dr. Kon is a recipient of the Established Investigator Award from the American Heart Association.

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